IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Cantor *et al.*

Serial No.: 09/880,988

Confirmation No.: 5954

Filed: : June 13, 2001

For: *USE OF NUCLEOTIDE ANALOGS IN THE ANALYSIS OF OLIGONUCLEOTIDE MIXTURES AND IN HIGHLY MULTIPLEXED NUCLEIC ACID SEQUENCING*

Art Unit: 1634

Examiner: Chakrabarti, Arun K.

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ATTACHMENT TO THE AMENDMENT

1. Marked up copy of amended claims per 37 C.F.R. §1.121.

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MARKED UP CLAIMS (37 C.F.R. § 1.121)

Please amend claims Claims 4, 6, 17, 27, 28, 31, 33, 36 and 42 as follows:

4. (Amended) The method of claim 1, wherein a mass-matched deoxynucleotide is deoxyinosine, 5-nitroindole, 3-nitropyrrole , 3-methyl 7-propynyl isocarbostyril, 5-methyl [iscarbostyril] isocarbostyril or 3-methyl [iscarbostyril] isocarbostyril.

6. (Amended) The method of claim 5 that is a method for determining nucleotide sequences of a plurality of target nucleic [acids] acid molecules, comprising:

synthesizing extension products of the target nucleic acid in the presence of chain terminating nucleotides and mass-matched nucleotides;

determining the mass of each extension product; and

calculating a mass shift from a period for the mass of each extension product,

whereby the nucleotide sequences of the target nucleic acids are

determined by determining the nucleotide that corresponds to each mass shift.

17. (Amended twice) A method for detecting [a] one or a plurality of target nucleic acid(s) molecules or one or a plurality of nucleotides therein [molecules], comprising:

(a) copying the target nucleic acid molecule(s) in the presence of a pair-matched set of nucleotides;

(b) denaturing the resulting copies of the target(s) to produce single-stranded templates;

(c) annealing and ligating one or a plurality of partially duplex hairpin primers to the single-stranded template(s);

(d) extending the primer(s) in the presence of chain terminating nucleotides and pair-matched nucleotides to produce extension products, wherein the extension products follow a periodic mass distribution that is determined by the mass of the pair-matched nucleotide set; and

(e) detecting each of the targets or nucleotides therein by virtue of the mass shift of each extension product from its corresponding periodic reference mass.

27. (Amended) A method for detecting a plurality of target nucleic acid molecules in a sample containing nucleic acid molecules, comprising:

preparing a composition containing a plurality of pair-matched nucleic acid molecules or mass-matched nucleic acid molecules from a sample comprising the target nucleic acid molecules;

analyzing the resulting composition by mass spectrometry; and

detecting target nucleic acid molecules.

28. (Amended) A process for detecting a mutation in a target nucleic acid sequence in a target nucleic acid molecule, in a sample, comprising:

a) hybridizing [a nucleic acid molecule] a primer to nucleic acid molecules in the sample, thereby producing a hybridized primer and a molecule from the sample, wherein:

the nucleic molecules from the sample are optionally immobilized[;] and

the primer is complementary to a sequence in the target nucleic acid sequence that is adjacent to the region suspected of containing a mutation sequence;

b) contacting the hybridized primer with a composition comprising mass-matched deoxyribonucleoside triphosphates and a chain terminating nucleotide selected from a dideoxyribonucleoside triphosphate or a 3'-deoxynucleoside triphosphate and optionally one or more deoxyribonucleoside triphosphates, such that the hybridized primer is extended until a chain terminating nucleotide is incorporated, thereby producing an extended primer; and

c) determining the mass of the extended primer, thereby determining whether a mutation is present in the target nucleic acid sequence.

31. (Amended) A process for detecting mutations in a plurality of target nucleic acid sequences in a sample, comprising:

a) hybridizing a plurality of primers to nucleic acid molecules in the sample, thereby producing [a] hybridized primers, wherein:

the nucleic acid molecules from the sample are optionally immobilized[;] and each primer is complementary to a sequence of a target nucleic acid sequence that is adjacent to a region suspected of containing a mutation sequence;

b) contacting the hybridized primers with a composition comprising a chain terminating nucleotide selected from a mass-matched dideoxyribonucleoside triphosphate or a 3'-deoxynucleoside triphosphate and one or more deoxyribonucleoside triphosphates, such that the hybridized primers are extended until a chain terminating nucleotide is incorporated, thereby producing an extended primer; and

c) determining the mass of the extended primers, thereby

determining whether mutations are present in the target nucleic acid sequences.

33. (Amended) The method of claim 31, wherein the [mass] masses of the extended primers are determined by mass spectrometry.

36. (Amended twice) A method for detecting a plurality of target nucleic acid sequences, comprising the steps of:

a) hybridizing a primer or plurality thereof to nucleic acid molecules comprising target nucleic acid sequences, wherein the primers can be extended in a 3' direction towards the target nucleic acid sequence, and wherein the 5' end of the hybridized mass-matched nucleic acid molecules can be selectively cleaved from the extension product;

b) extending the primers in the presence of mass matched deoxyribonucleotides and a polymerase to produce extension products;

c) selectively cleaving the 5' end of the primers from the extension products to produce portions of the primers and cleaved extension products; and

d) detecting the cleaved extension products.

42. (Amended) A method for detecting a target nucleic acid sequence, comprising:

a) hybridizing first and second primers to a nucleic acid molecule containing the target nucleic acid sequence, wherein a primer contains a selectively cleavable site at its 3' end;

b) extending the primers in the presence of mass-matched nucleotides;

c) cleaving the resulting product at the selectively cleavable sites; and

d) analyzing the masses of the cleavage products, whereby the target sequence is detected.